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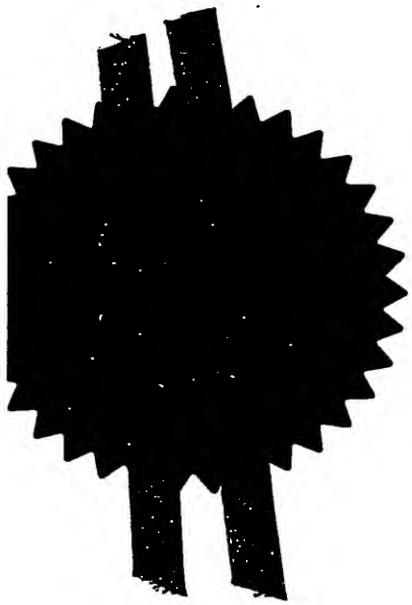

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CAMBRIDGE UNIVERSITY TECHNICAL SERVICES LIMITED
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Cambridge CB2 1TS
United Kingdom

Patents ADP number *(if you know it)* 6958809004

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention — Biomaterial

5. Name of your agent *(if you have one)* — BOULT WADE TENNANT

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

VERULAM GARDENS
70 GRAY'S INN ROAD
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Patents ADP number *(if you know it)* 42001

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Signature: R. Setna, Boult Wade Tennant

Date: 28 October 2003

12. Name and daytime telephone number of person to contact in the United Kingdom: Rohan Setna

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Biomaterial

The present invention relates to the field of synthetic bone, dental materials and regeneration scaffolds for biomedical applications and, in particular, to synthetic bone, dental materials and regeneration scaffolds and their precursors comprising: collagen, a calcium phosphate material and one or more glycosaminoglycans.

Natural bone material comprises apatite, the composition of which is similar to hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. The similarity of hydroxyapatite to natural bone material has resulted in the development of synthetic biomedical materials that comprise hydroxyapatite. The challenge faced by researchers in the field is to make a synthetic material that has a composition and structure that will allow natural bone growth in and around the synthetic material in the human or animal body.

It has been observed that bone will bond directly to hydroxyapatite in the human body (a property referred to as bioactivity) through a bone-like apatite layer formed in the body environment.

Hydroxyapatite is a relatively insoluble material when compared to other forms of calcium phosphate materials such as brushite, tricalcium phosphate and octacalcium phosphate. The relatively low solubility of apatite can be a disadvantage when producing a biomaterial as the rate of resorption of the material in the body is particularly slow.

Hydroxyapatite is a mechanically strong material. However, the material is relatively brittle when compared to natural bone.

Previous attempts in the prior art of producing a synthetic bone-substitute material having improved mechanical strength over hydroxyapatite include combining collagen and apatite by mechanical mixing. Such a mechanical method is described in EP-A-0164 484.

Later developments in the technology include producing a bone-replacement material comprising hydroxyapatite, collagen and chondroitin-4-sulphate by the mechanical mixing of these components. This is described in EP-A-0214070. This document further describes dehydrothermic crosslinking of the chondroitin-4-sulphate to the collagen. Materials comprising apatite, collagen and chondroitin-4-sulphate have been found to have good biocompatibility. The mechanical mixing of the apatite with the collagen, and optionally chondroitin-4-sulphate, essentially forms collagen/chondroitin-4-sulphate-coated particles of apatite. It has been found that such a material, although biocompatible, produces limited in-growth of natural bone when in the human or animal body and no remodeling of the calcium phosphate phase of the synthetic material.

The present invention thus seeks to address at least some of the problems associated with the prior art.

In a first aspect, the present invention provides a process for the production of a composite material

comprising collagen, brushite and one or more glycosaminoglycans, said process comprising the steps of

providing an acidic aqueous solution comprising collagen, a calcium source and a phosphorous source and one or more glycosaminoglycans, and

precipitating the collagen, the brushite and the one or more glycosaminoglycans together from the aqueous solution to form a triple co-precipitate.

In a second aspect, the present invention provides a process for the production of a composite biomaterial comprising collagen, octacalcium phosphate and one or more glycosaminoglycans, wherein said method comprises the steps of

providing a composite material comprising collagen, brushite and one or more glycosaminoglycans, and

converting at least some of the brushite in the composite material to octacalcium phosphate by hydrolysation.

In a third aspect, the present invention provides a process for the production of a composite biomaterial comprising collagen, apatite and one or more glycosaminoglycans, wherein said process comprises the steps of

providing a composite material comprising collagen, brushite and one or more glycosaminoglycans, and

converting at least some of the brushite in the composite material to apatite by hydrolysation.

In a fourth aspect, the present invention provides a precursor material suitable for transforming into a

synthetic biomaterial, said precursor material comprising a composite material comprising collagen, brushite and one or more glycosaminoglycans.

In a fifth aspect, the present invention provides a composite biomaterial comprising collagen, brushite and one or more glycosaminoglycans, said composite biomaterial obtainable by the process of the present invention.

In a sixth aspect, the present invention provides a composite biomaterial comprising brushite, one or more glycosaminoglycans and collagen.

In a seventh aspect, the present invention provides a composite biomaterial comprising collagen, octacalcium phosphate and one or more glycosaminoglycans.

In an eighth aspect, the present invention provides a biomaterial comprising particles of one or more calcium phosphate materials, collagen and one or more glycosaminoglycans, wherein said collagen and said one or more glycosaminoglycans are crosslinked and form a matrix, said particles of calcium phosphate material are dispersed in said matrix of collagen and one or more glycosaminoglycans and said calcium phosphate material is selected from brushite, octacalcium phosphate and apatite.

A "triple co-precipitate" includes the precipitate of the three compounds, wherein all compounds have been precipitated at substantially the same time from the same solution/dispersion. It is to be distinguished from a

material formed from the mechanical mixing of the components and particularly where these components have been precipitated separately, for instance in different solutions. The microstructure of a co-precipitate is typically substantially different from a material formed from the mechanical mixing of its components.

In the first aspect of the present invention, the solution preferably has a pH of from 2.5 to 5.5. More preferably, the solution has a pH of from 3.0 to 3.8. Still more preferably, the solution has a pH of from 3.2 to 3.3. Most preferably, the solution has a pH of around 3.2.

The calcium source is preferably selected from one or more of calcium nitrate, calcium acetate, calcium chloride, calcium carbonate, calcium alkoxide, calcium hydroxide, calcium silicate, calcium sulphate, calcium gluconate and the calcium salt of heparin. A calcium salt of heparin may be derived from the porcine intestinal mucosa. Suitable calcium salts are commercially available from Sigma-Aldrich Inc.

The phosphorus source is preferably selected from one or more of ammonium-dihydrogen phosphate, diammonium hydrogen phosphate, phosphoric acid, disodium hydrogen orthophosphate 2-hydrate ($Na_2HPO_4.2H_2O$, sometimes termed GPR Sorensen's salt) and trimethyl phosphate, alkali metals salts (e.g Na or K) of phosphate, alkaline earth salts (e.g. Mg or Ca) of phosphate.

Glycosaminoglycans are a family of macromolecules containing long unbranched polysaccharides containing a

repeating disaccharide unit. Preferably, the one or more glycosaminoglycans are selected from chondroitin sulphate, dermatin sulphate, heparin, heparin sulphate, keratin sulphate and hyaluronic acid. Chondroitin sulphate may be chondroitin-4-sulphate or chondroitin-6-sulphate, both of which are available from Sigma-Aldrich Inc. The chondroitin-6-sulphate may be derived from shark cartilage. Hyaluronic acid may be derived from human umbilical chord. Heparin may be derived from porcine intestinal mucosa.

Preferably, in the precipitation of the triple co-precipitate, the solution has a temperature of from 4.0 to 50 degrees centigrade. More preferably, the solution has a temperature of from 15 to 40 degrees centigrade. The solution may be at room temperature, that is between 20 and 30 degrees centigrade.

The concentration of calcium ions is preferably from 0.0167 to 1.67 $moldm^{-3}$, more preferably 0.08 to 0.25 $moldm^{-3}$, still more preferably approximately 0.167 $moldm^{-3}$.

Preferably, the ratio of collagen to the total amount of one or more glycosaminoglycans in the solution prior to precipitation is from 8:1 to 30:1 by weight. More preferably, the ratio of collagen to the total amount of one or more glycosaminoglycans in the solution prior to precipitation is from 10:1 to 12:1 by weight. Most preferably, the ratio of collagen to the total amount of one or more glycosaminoglycans in the solution prior to precipitation is from 11:1 to 23:2 by weight.

Preferably, the ratio of collagen to brushite in the triple co-precipitate is from 100:1 to 1:10 by weight, more preferably from 3:1 to 1:6 by weight, most preferably from 3:2 to 1:4 by weight.

Preferably, the concentration of collagen in the solution prior to precipitation is from 1.0g/L to 10.0g/L. Preferably, the concentration of collagen in the solution prior to precipitation is from 1.5g/L to 2.5g/L, most preferably 1.5g/L to 2.0g/L, where the process includes the steps of filtration/low temperature drying. Preferably, the concentration of collagen in the solution prior to precipitation is from 5g/L to 7.5g/L, most preferably 6.0g/L to 7.2g/L, where the process include the steps of other means of shaping such as freeze drying and injection moulding.

Preferably, the total concentration of the one or more glycosaminoglycans in the solution prior to precipitation is from 0.01g/L to 1.0g/L, more preferably 0.4g/L to o.6g/L, and most preferably 0.5g/L to 0.55g/L.

Preferably the solution comprises calcium ions and the ratio of collagen to the calcium ions is from 100:1 to 1:20 by weight, more preferably 3:2 to 1:3 and most preferably 3:2 to 1:2.

Preferably, the ratio of collagen to brushite in the material is from 100:1 to 1:10 by weight, more preferably 3:1 to 1:6 and most preferably 3:2 to 1:4.

Preferably, the solution comprises calcium ions and the concentration of calcium ions in solution is from 0.00025M to 0.5M, more preferably 0.05 to 0.40M and most preferably 0.10 to 0.35M.

Preferably, the solution comprises phosphate ions and the concentration of phosphate ions in solution is from 0.00025M to 0.50M, more preferably 0.05 to 0.40M, most preferably 0.10 to 0.35.

Preferably, the total concentration of the one or more glycosaminoglycans in the solution is from 0.01g/L to 1.0g/L more preferably 0.40g/L to 0.60g/L and most preferably 0.50g/L to 0.55g/L.

Precipitation may be effected by combining the collagen, the calcium source, the phosphorous source and one or more glycosaminoglycans in an acidic aqueous solution and either allowing the solution to stand until precipitation occurs, agitating the solution, titration using basic titrants such as ammonia, addition of a nucleating agent such as pre-fabricated brushite, varying the rate of addition of the calcium source, and any combination of these techniques.

In a second aspect, the present invention provides a process for the production of a composite biomaterial comprising collagen, octacalcium phosphate and one or more glycosaminoglycans, wherein said method comprises the steps of

providing a composite material of collagen, brushite and one or more glycosaminoglycans, and

converting at least some of the brushite in the composite material to octacalcium phosphate by hydrolysation.

"Biomaterial" means a material that is biocompatible with a human or animal body.

Preferably, the composite material comprises or consists essentially of a triple co-precipitate comprising collagen, brushite and one or more glycosaminoglycans. The triple co-precipitate may be formed by a process as defined in the first aspect of the invention above.

Preferably, the step of hydrolysation of brushite to octacalcium phosphate comprises contacting the triple co-precipitate with an aqueous solution, said aqueous solution being at or above the pH at which octacalcium phosphate becomes thermodynamically more stable than brushite. Preferably, this aqueous solution has a pH of from 6 to 8.

More preferably, this aqueous solution has a pH of from 6.5 to 7.5. Most preferably, this aqueous solution has pH of about 6.65.

In a third aspect, the present invention provides a process for the production of a composite biomaterial comprising collagen, apatite and one or more glycosaminoglycans, wherein said process comprises the steps of

providing a composite material of collagen, brushite and one or more glycosaminoglycans, and

converting at least some of the brushite in the composite material to apatite by hydrolysation.

Preferably, the composite material comprises or consists essentially of a triple co-precipitate comprising collagen, brushite and one or more glycosaminoglycans.

The triple co-precipitate may be formed according to the process defined in the first aspect of the present invention.

Preferably, the step of hydrolysation of brushite to apatite comprises contacting the triple co-precipitate with an aqueous solution, said aqueous solution being at or above the pH at which apatite becomes thermodynamically more stable than brushite. Preferably for the conversion of brushite to apatite, the aqueous solution has a pH of 8.0 or more.

"Apatite" is a class of minerals comprising calcium and phosphate and has the general formula: $Ca_5(PO_4)_3(X)$, wherein X may be an ion that is typically OH^-, F^- and Cl^-, as well as other ions known to those skilled in the art. "Apatite" also includes substituted apatites such as silicon-substituted apatites. "Apatite" includes hydroxyapatite, which is a specific example of an apatite. The hydroxyapatite may also be substituted with silicon.

Methods of increasing the rate of conversion of brushite to octacalcium phosphate and/or apatite include increasing the temperature, the brushite concentration in solution and the agitation speed.

It may be desirable to produce a biomaterial according to the present invention comprising both apatite and octacalcium phosphate. The processes of the second and

third aspects of the present invention may be combined to produce a material comprising both octacalcium phosphate and apatite. The brushite in the triple co-precipitate may first be converted to octacalcium phosphate and then the octacalcium phosphate may be partially converted to apatite. Total, or near total (i.e. at least 98%), conversion of brushite or octacalcium phosphate to apatite typically occurs by hydrolysation at a pH of 8.0 or more for a period of about 12 hours. Partial conversion of the brushite and/or apatite in the material may therefore be effected by hydrolysation for a period of less than 12 hours.

In the second or third aspect of the present invention, the conversion of brushite to octacalcium phosphate and/or apatite is preferably conducted at a temperature of from 30 to 40 degrees centigrade. More preferably, the conversion is conducted at a temperature of from 36 to 38 degrees centigrade. Most preferably, the conversion is conducted at a temperature of about 37 degrees centigrade.

Preferably, the processes of the present invention comprise the step of crosslinking the one or more glycosaminoglycans and the collagen in the triple co-precipitate. By triple co-precipitate this includes the triple co-precipitate comprising collagen, brushite and one or more glycosaminoglycans, and the co-precipitate's derivatives. Its 'derivatives' include the co-precipitate wherein at least some of the brushite has been converted to octacalcium phosphate and/or apatite, and the co-precipitate that has been shaped or moulded, or subjected to any further chemical or mechanical processing.

Preferably, at least some of the brushite is converted to octacalcium phosphate and/or apatite, the glycosaminoglycan and collagen are crosslinked prior to the conversion of the brushite to octacalcium phosphate and/or apatite. This crosslinking may be effected by subjecting the triple co-precipitate to gamma radiation and/or ultraviolet radiation, non-enzymatic glycation with a simple sugar such as glucose, mannose, ribose and sucrose, contacting the triple co-precipitate with glutaraldehyde and/or ethyl dimethylaminopropyl carbodiimide or any combination of these methods.

Preferably, if at least some of the brushite is converted to octacalcium phosphate and/or apatite, the glycosaminoglycan and collagen are crosslinked subsequent to the conversion of the brushite to octacalcium phosphate and/or apatite. The crosslinking subsequent to the conversion of the brushite to apatite/octacalcium phosphate may be effected by the methods mentioned above and in addition by a dehydrothermal treatment. Dehydrothermal treatment includes subjecting a substrate to a low pressure atmosphere at a raised temperature. The temperature in the dehydrothermal treatment may be of from 95°C to 135°C. The temperature may preferably be of from 100°C to 110°C, and most preferably of from 105°C to 110°C, if completion of the dehydrothermal treatment is desired in typically 18 to 36 hours. The temperature may preferably be of from 120°C to 135°C, and most preferably of from 125°C to 135°C, if completion of the dehydrothermal treatment is desired in typically 4 to 8 hours.

Preferably, the collagen and the glycosaminoglycan are crosslinked both prior to and subsequent to conversion of the brushite to octacalcium phosphate and/or apatite.

The processes of the present invention may comprise the step of shaping the composite biomaterial into a structure suitable for use as a bone or dental substitute. Such a step may occur after formation of the triple co-precipitate, but prior to any conversion of the brushite or crosslinking of the collagen and glycosaminoglycan that may occur.

Alternatively, the step of shaping the biomaterial may occur subsequent to either the conversion of the brushite to apatite and/or octacalcium phosphate or crosslinking of the collagen and the glycosaminoglycan.

Preferably, the composite material is shaped using a technique selected from (i) filtration and low temperature drying, (ii) freeze drying, (iii) injection moulding and (iv) cold pressing. Filtration and low temperature drying, wherein the temperature is from 15°C to 40°C, most preferably of from 35°C to 40°C, typically results in a dense granular form of material. Freeze drying typically results in an open porous form. Injection moulding results in a wide variety of shapes/morphologies of a material depending on the shape of the dye used. Cold pressing typically results in a dense pellet form.

The present invention provides a precursor material suitable for transforming into a synthetic biomaterial, said precursor material comprising a composite material comprising collagen, brushite and one or more

glycosaminoglycans. Preferably, the composite material comprises or consists essentially of a triple co-precipitate comprising collagen, brushite and one or more glycosaminoglycans. The triple co-precipitate may be produced according to a process of the first aspect of the present invention.

The present invention provides a composite biomaterial comprising collagen, one or more glycosaminoglycans and brushite obtainable by any of the processes of the present invention.

The present invention provides a composite biomaterial comprising collagen, octacalcium phosphate and one or more glycosaminoglycans obtainable by the process of the second aspect of the present invention.

The present invention provides a composite biomaterial comprising collagen, apatite and one or more glycosaminoglycans obtainable by the process of the third aspect of the present invention.

The present invention provides a composite biomaterial of the present invention suitable for use as a substitute bone or dental material.

The present invention provides a composite biomaterial comprising a triple co-precipitate of collagen, glycosaminoglycan and brushite.

Preferably the collagen and the one or more glycosaminoglycans have been crosslinked in the material(s) of the present invention.

Preferably, the collagen is present in the material of the present invention in an amount of from 10 to 99wt%, more preferably 20 to 40 wt%.

Preferably, the one or more glycosaminoglycans are present in the material of the present invention in an amount of from 0.01 to 12wt%, more preferably from 3 to 8 wt %.

Preferably, if the material comprises brushite, the ratio of collagen to brushite is 100:1 to 1:10 by weight, more preferably 3:1 to 1:6 by weight, most preferably 3:2 to 1:4 by weight.

Preferably if the material comprises octacalcium phosphate, the ratio of collagen to octacalcium phosphate is 100:1 to 1:10 by weight, more preferably 3:1 to 1:6 by weight, most preferably 3:2 to 1:4 by weight.

Preferably, the ratio of collagen to the total amount of one or more glycosaminoglycans is from 10:1 to 30:1 by weight, more preferably 10:1 to 12:1, most preferably 11:1 to 23:2.

The present invention also provides a synthetic bone material, bone implant, bone graft, , bone scaffold, soft tissue regeneration scaffold, skin regeneration scaffold, neural tissue regeneration scaffold, filler, coating or

cement comprising a composite biomaterial of the present invention. By coating, this term includes any coating comprising the biomaterial or precursor of the present invention. The coating may be applied to the external or internal surfaces of prosthetic members, bones, or any substrate intended for use in the human or animal body, which includes particulate materials. The composition of the present invention may be used for both in-vivo and ex-vivo repair of both mineralized biological material, including but not limited to bone and dental materials, and non-mineralised material, including but not limited to, cartilage, tissue comprising scaffold proteins such as collagen, fibrinogen, fibronectin, cells including but not limited to animal cells including human cells, endothelial cells, neural cells, muscle cells, renal cells, cells and skin cells. Cell types are well known in the art and include many specific cell types. The biomaterials of the present invention may be used in the growth of allografts and autografts.

The biomaterial of the present invention that comprises octacalcium phosphate may by free or essentially free (i.e. comprising less than 2% by weight of brushite in total amount of calcium phosphate materials in the biomaterial) of any of the precursor brushite phase of material. The calcium phosphate material may comprise or consist essentially of phase pure octacalcium phosphate or apatite. By phase pure, this means preferably containing at least 98%, more preferably at least 99%, and most preferably, at least 99.5% of the desired phase. Alternatively, the biomaterial may comprise a mixture of octacalcium phosphate

and apatite, depending on the desired properties of the biomaterial.

The material of the present invention comprising brushite may be used either as a precursor material for making a biomaterial, or may be suitable in itself for use as a biomaterial.

Examples

The following experiments are provided by way of example, and are not to be considered limiting to the scope of the invention.

The starting materials used in the following Example process are as follows:

Collagen: reconstituted, pepsin-extracted porcine dermal collagen (atelocollagen); 85% by weight of Type I, 15% by weight of Type III; Japan Meat Packers (Osaka, Japan) GAG.

Chondroitin-6-sulphate from shark cartilage; sodium salt; Sigma-Aldrich Inc (St. Louis, MO, USA).

Calcium Sources:

i) Calcium hydroxide; $Ca(OH)_2$ Sigma-Aldrich Inc (St. Louis, MO, USA);and

ii) Calcium nitrate; $Ca(NO_3)_2.4H_2O$; Sigma-Aldrich Inc (St. Louis, MO, USA).

Phosphorous Source:

Orthophosphoric acid; H_3PO_4; BDH Laboratory Supplies (Poole, United Kingdom)

Titrant: Ammonia; NH_3; BDH Laboratory Supplies (Poole, United Kingdom):

The procedure used to make the Example biomaterial was as follows:

Step I

Solution A was prepared by dissolving $Ca(OH)_2$ in 0.48M H_3PO_4 to a concentration of 0.12M at room temperature, and the resulting solution titrated to pH of 3.2.

Suspension B was prepared by dissolving Chondroitin-6-sulphate in deionised water to a concentration of 3.2g/L. Under constant stirring, $Ca(NO_3)_2.4H_2O$ and $Ca(OH)_2$ then added to chondroitin sulphate solution at a nitrate:hydroxide molar ratio of 1.5, to produce a suspension with a total calcium concentration of 2.4M.

0.144g collagen were added to 20mL of Solution A, and blended using a homogeniser until dissolved. 4mL of Suspension B was then added to Solution A under constant stirring. Stirring was continued for 60 minutes, and pH monitored to ensure that it remained in the range $3.15<pH<3.30$. The resulting slurry was then allowed to age for 24 hours at room temperature.

Step II

The slurry was allowed to dry at 37°C in air for 5 days, and the remaining triple co-precipitate rinsed with deionised water, and subsequently dried again at 37°C for an additional 24 hours.

Step III

Co-precipitates were placed in dilute acetic acid (pH = 3.2), and irradiated with a gamma irradiation dose of 30kGy. The crosslinked precipitates were then removed from solution, rinsed, and dried at 37°C in air.

Step IV

Crosslinked, co-precipitate granules were placed in 50mL deionised water at 37°C, and the pH of the solution adjusted to 6.65 using ammonia. Temperature and pH were maintained constant for 48 hours, after which the co-precipitates were filtered, rinsed in deionised water, and dried at 37°C in air.

Step V

Crosslinked, hydrolysed, co-precipitate granules were placed in a vacuum oven at room temperature, and a vacuum of 50mTorr applied, after which the temperature was then increased to 105°C. After 24 hours, the temperature was reduced to room temperature and the vacuum released.

Analysis:-

Figure 1 shows the x-ray diffraction pattern of the composite immediately following triple co-precipitation and drying (Steps I and II). This pattern confirms the major phase present to be brushite.

Figure 2 shows an SEM micrograph of the structure of co-precipitate granules following primary crosslinking (Step III). It is worthy to note the microstructurally homogeneous nature of the granules.

The progression of hydrolysis to octacalcium phosphate (Step IV) is illustrated in the XRD Pattern of Figure 3. Progressive decreases in the intensity of the brushite peak at 12.5°, and increases of the major octacalcium

phosphate(OCP) peak at 4.5° indicate the conversion of the inorganic phase to OCP over a period of 48 hours.

A TEM image of the composite is shown in Figure 4. A random distribution of 10-20nm low aspect-ratio calcium phosphate crystals dispersed in a collagen/GAG matrix is evident.

The advantages and purpose of the various steps that may be employed in the present invention are described below

I: Triple Co-precipitation of Collagen, GAG, and Brushite at Acidic pH

This step is performed to initiate simultaneous formation, via precipitation from solution, of the three constituents of this composite, and to control the ratios of the three respective phases.

One may control the compositional properties of the composite (and in particular the collagen:GAG:CaP ratio) by variation of pH, temperature, ageing time, calcium ion concentration, phosphorous ion concentration, collagen concentration and GAG concentration.

The pH may be maintained constant (using buffers, pH-stat titration or other methods), or allowed to vary The possible secondary (contaminant) phases that may form include other acidic calcium phosphates (e.g. monetite, calcium hydrogen phosphate), and complexes including by-products of titration and reactant addition (e.g. ammonium phosphate, ammonium nitrate).

Additives to aid in crosslinking (e.g. glucose, ribose) or to enhance in-vivo response (e.g. growth factors, gene transcription factors, silicon, natriuretic peptides etc.) may also be added during this step

II: Net Shape Formation

This step may be performed to produce the desired architecture of the final composite form, with particular emphasis on control of pore architecture

III: Primary Crosslinking (crosslinking prior to conversion of brushite to octacalcium phosphate and/or apatite)

This step may be performed to ideally produce a material that, when placed in a solution of elevated pH, the GAG content of the composite does not elude rapidly, and, furthermore, to enhance the mechanical and degradation properties of the composite
In addition, these steps may ensure that the brushite phase does not convert to monetite (a phase of calcium phosphate that cannot be converted to Octacalcium phosphate or Hydroxyapatite), this step must preferably be performed at a temperature of no greater than 37°C

IV) Hydrolysis

This step may be performed to partially or fully hydrolyse the calcium phosphate phase from brushite (phase with high solubility at physiological pH) to octacalcium phosphate and/or apatite (phases with lower solubility at

physiological pH), and to remove any soluble contaminant phases (e.g. ammonium nitrate, calcium hydrogen phosphate)

In the case of hydrolysis to OCP, the selected pH may preferably be maintained constant at approximately 6.65 (using a buffer, pH stat, or other method), and the temperature at approximately 37°C for 24-48 hours.

As was the case in Step I, additives to aid in crosslinking (e.g. glucose, ribose) or to enhance in-vivo response (e.g. growth factors, gene transcription factors, silicon, natriuretic peptides etc.) may also be added during the hydrolysis step (Step IV).

V) Secondary Crosslinking (crosslinking subsequent to conversion of brushite to octacalcium phosphate and/or apatite)

This step may be performed to further tailor the mechanical and degradation properties of the composite. Provided that substantially all of the brushite has been converted to OCP or hydroxyapatite (Hap) (Step IV), in addition to the techniques listed in Step III above, dehydrothermal treatment may also be applied during secondary crosslinking (Step V).

The composite biomaterials of the present invention may be used as a bioresorbable material. Following implantation, it is expected that a device fabricated from the material would resorb completely, leaving behind only healthy, regenerated tissue, with no remaining trace of the implant itself.

Claims:

1. A process for the production of a composite material comprising collagen, brushite and one or more glycosaminoglycans, said process comprising the steps of

providing an acidic aqueous solution comprising collagen, a calcium source and a phosphorous source and one or more glycosaminoglycans, and

precipitating the collagen, the brushite and the one or more glycosaminoglycans together from the aqueous solution to form a triple co-precipitate.

2. A process as claimed in claim 1, wherein the solution has a pH of from 2.5 to 5.5

3. A process as claimed in claim 2, wherein the solution has a pH of from 3 to 3.8.

4. A process as claimed in claim 3, wherein the solution has a pH of about 3.2.

5. A process as claimed in any one of the preceding claims, wherein the calcium source is selected from one or more of calcium nitrate, calcium acetate, calcium chloride, calcium carbonate and calcium alkoxide, calcium hydroxide, calcium silicate, calcium sulphate, calcium gluconate and the calcium salt of heparin.

6. A process as claimed in any one of the preceding claims, wherein the phosphorus source is selected from one or more of ammonium-dihydrogen phosphate, diammonium

hydrogen phosphate, phosphoric acid, disodium hydrogen orthophosphate 2-hydrate and trimethyl phosphate.

7. A process as claimed in any one of the preceding claims, wherein the one or more glycosaminoglycans are selected from chondroitin sulphate, dermatin sulphate, heparin, heparin sulphate, keratin sulphate and hyaluronic acid.

8. A process as claimed in any one of the preceding claims, wherein the solution has a temperature of from 4 to 50 degrees centigrade.

9. A process as claimed in any one of the preceding claims, wherein the solution has a temperature of from 15 to 40 degrees centigrade.

10. A process as claimed in any one of the preceding claims wherein the ratio of collagen to the total amount of one or more glycosaminoglycans in the solution is from 8:1 to 30:1 by weight.

11. A process as claimed in any one of the preceding claims, wherein the solution comprises calcium ions and the ratio of collagen to the calcium ions is from 100:1 to 1:20 by weight.

12. A process as claimed in any one of the preceding claims, wherein ratio of collagen to brushite in the co-precipitate is from 100:1 to 1:10 by weight.

13. A process as claimed in any one of the preceding claims wherein the solution comprises calcium ions and the concentration of calcium ions in solution is from 0.00025M to 0.5M.

14. A process as claimed in any one of the preceding claims wherein the solution comprises phosphate ions and the concentration of phosphate ions in solution is from 0.00025M to 0.50M.

15. A process as claimed in any one of the preceding claims wherein the concentration of collagen in the solution is from 1.0g/L to 10g/L.

16. A process as claimed in any one of the preceding claims wherein the total concentration of the one or more glycosaminoglycans in the solution is from 0.01g/L to 1.0g/L.

17. A process for the production of a composite biomaterial comprising collagen, octacalcium phosphate and one or more glycosaminoglycans , wherein said method comprises the steps of

providing a composite material comprising collagen, brushite and one or more glycosaminoglycans, and

converting at least some of the brushite in the composite material to octacalcium phosphate by hydrolysation.

18. A process as claimed in claim 17, wherein the composite material comprises or consists essentially of a triple co-

precipitate comprising collagen, brushite and one or more glycosaminoglycans.

19. A process as claimed in claim 18, wherein the triple co-precipitate is formed according to the process of any one of claims 1 to 16.

20. A process as claimed in any one of claims 17 to 19, wherein the step of hydrolysation of brushite to octacalcium phosphate comprises contacting the composite material with an aqueous solution, said aqueous solution being at or above the pH at which octacalcium phosphate becomes thermodynamically more stable than brushite.

21. A process as claimed in claim 20, wherein said aqueous solution has a pH of from 6 to 8.

22. A process as claimed in claim 21, wherein said aqueous solution has a pH of from 6.5 to 7.5.

23. A process as claimed in claim 22, wherein said aqueous solution has pH of about 6.65.

24. A process for the production of a composite biomaterial comprising collagen, apatite and one or more glycosaminoglycans, wherein said process comprises the steps of

providing a composite material comprising collagen, brushite and one or more glycosaminoglycans, and

converting at least some of the brushite in the composite material to apatite by hydrolysation.

25. A process as claimed in claim 24, wherein the composite material comprises or consists essentially of a triple co-precipitate comprising collagen, brushite and one or more glycosaminoglycans.

26. A process as claimed in claim 25, wherein the triple co-precipitate is formed according to any one of claims 1 to 16.

27. A process as claimed in any one of claims 24 to 26, wherein the step of hydrolysation of brushite to apatite comprises contacting the composite material with an aqueous solution, said aqueous solution being at or above the pH at which apatite becomes thermodynamically more stable than brushite.

28. A process as claimed in claim 27, wherein said aqueous solution has a pH of 8.0 or more.

29. A process as claimed in any one of claims 17 to 28, wherein the conversion of brushite to octacalcium phosphate and/or apatite is at a temperature of from 30 to 40 degrees centigrade.

30. A process as claimed in claim 21, wherein said temperature is from 36 to 38 degrees centigrade.

31. A process as claimed in claim 30, wherein said temperature is about 37 degrees centigrade.

32. A process as claimed in any one of the preceding claims further comprising the steps of crosslinking the collagen

and the one or more glycosaminoglycans in the composite material or triple co-precipitate.

33. A process as claimed in claim 32, wherein, if at least some of the brushite is converted to octacalcium phosphate and/or apatite, the glycosaminoglycan is crosslinked prior to the conversion of the brushite to octacalcium phosphate and/or apatite.

34. A process as claimed in claim 33, wherein the crosslinking is effected by one or more of: subjecting the triple co-precipitate to gamma radiation and/or ultraviolet radiation, non-enzymatic glycation with a simple sugar such as glucose, mannose, ribose and sucrose contacting the triple co-precipitate with glutaraldehyde and/or ethyl dimethylaminopropyl carbodiimide.

35. A process as claimed in any one of claims 32 to 34, wherein, if at least some of the brushite is converted to octacalcium phosphate and/or apatite, the collagen and one or more of glycosaminoglycans are crosslinked subsequent to the conversion of the brushite to octacalcium phosphate and/or apatite.

36. A process as claimed in claim 35, wherein the crosslinking is effected by one or more of: subjecting the triple co-precipitate to gamma radiation, ultraviolet radiation or dehydrothermic treatment, non-enzymatic glycation with a simple sugar such as glucose, mannose, ribose and sucrose, contacting the triple co-precipitate with glutaraldehyde or ethyl dimethylaminopropyl carbodiimide.

37. A process as claimed in any one of claims 32 to 36, wherein the collagen and the one or more glycosaminoglycan are crosslinked both prior to and subsequent to conversion of the brushite to octacalcium phosphate and/or apatite.

38. A process as claimed in any one of the preceding claims, further comprising the step of shaping the composite biomaterial into a structure suitable for use as a bone or dental substitute.

39. A process as claimed in any claim 38, wherein the composite material is shaped using a technique selected from filtration and low temperature drying, freeze drying, injection moulding and cold pressing.

40. A precursor material suitable for transforming into a synthetic biomaterial, said precursor material comprising a composite material comprising collagen, brushite and one or more glycosaminoglycans.

41. A precursor material as claimed in claim 40, wherein the composite material comprises or consists essentially of a triple co-precipitate comprising collagen, brushite and one or more glycosaminoglycans.

42. A precursor material as claimed in claim 41, wherein said triple co-precipitate is produced according to a process defined in any one of claims 1 to 16.

43. A composite biomaterial comprising collagen, one or more glycosaminoglycans and brushite obtainable by the process defined in any one of claims 1 to 39.

44. A composite biomaterial comprising collagen, octacalcium phosphate and one or more glycosaminoglycans obtainable by the process defined in any one of claims 17 to 39.

45. A composite biomaterial comprising collagen, apatite and one or more glycosaminoglycans obtainable by the process defined in any one of claims 24 to 39.

46. A composite biomaterial comprising brushite, one or more glycosaminoglycans and collagen.

47. A composite biomaterial comprising collagen, octacalcium phosphate and one or more glycosaminoglycans.

48. A composite biomaterial as claimed in any one of claims 43 to 47 suitable for use as a substitute bone or dental material.

49. A composite biomaterial comprising a triple co-precipitate of collagen, glycosaminoglycan and brushite.

50. A material as claimed in any one of claims 40 to 49, wherein the collagen and the one or more glycosaminoglycans have been crosslinked.

51. A material as claimed in any one of claims 40 to 50, wherein the collagen is present in the material in an amount of from 10wt% to 99wt%.

52. A material as claimed in any one of claims 40 to 51, wherein the one or more glycosaminoglycans are present in the material in an amount of from 0.01wt% to 12wt%.

53. A material as claimed in any one of claims 40 to 52, wherein if the material comprises brushite, the ratio of collagen to brushite is 100:1 to 1:10 by weight.

54. A material as claimed in any one of claims 40 to 52, wherein, if the material comprises octacalcium phosphate, the ratio of collagen to octacalcium phosphate is 100:1 to 1:10 by weight.

55. A material as claimed in any one of claims 40 to 54, wherein the ratio of collagen to the total amount of one or more glycosaminoglycans is from 10:1 to 30:1 by weight.

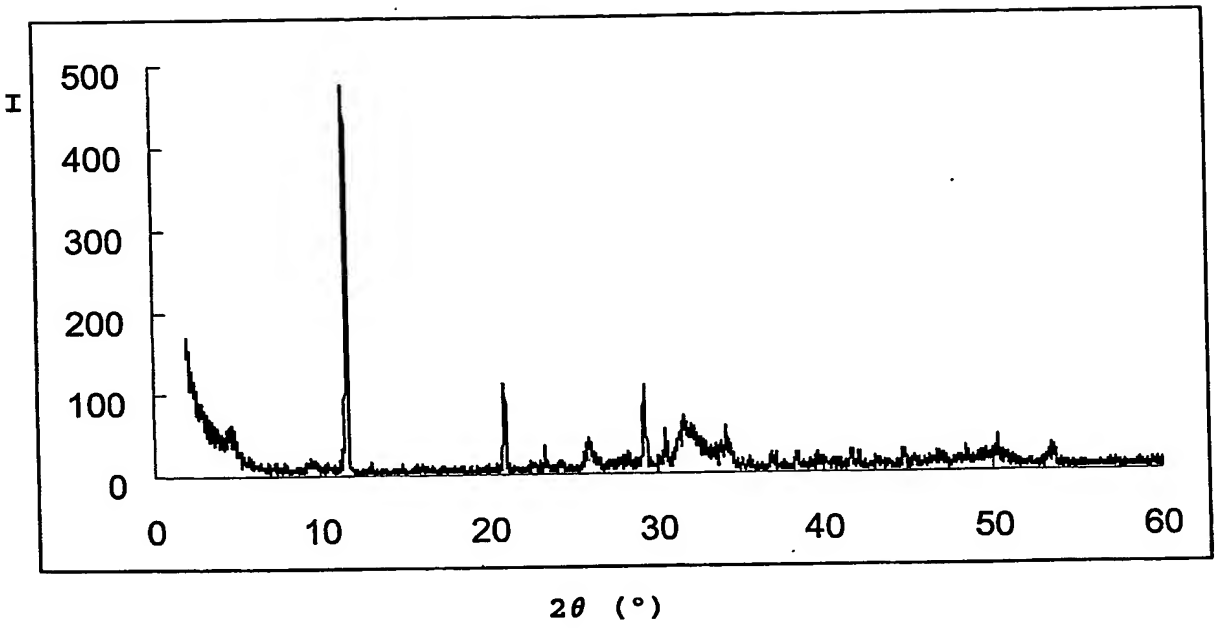
56. A biomaterial comprising particles of a calcium phosphate material, collagen and one or more glycosaminoglycans, wherein said collagen and said one or more glycosaminoglycans are crosslinked and form a matrix,

said particles of calcium phosphate material are dispersed in said matrix of collagen and one or more glycosaminoglycans, and

~~said calcium phosphate material is selected from~~ brushite and octacalcium phosphate.

57. A synthetic bone material, bone implant,

bone graft, bone scaffold, soft tissue regeneration scaffold, skin regeneration scaffold, neural tissue regeneration scaffold, filler, coating or cement comprising a material as claimed in any one of claims 43 to 56.



I = Relative Intensity

Figure 1

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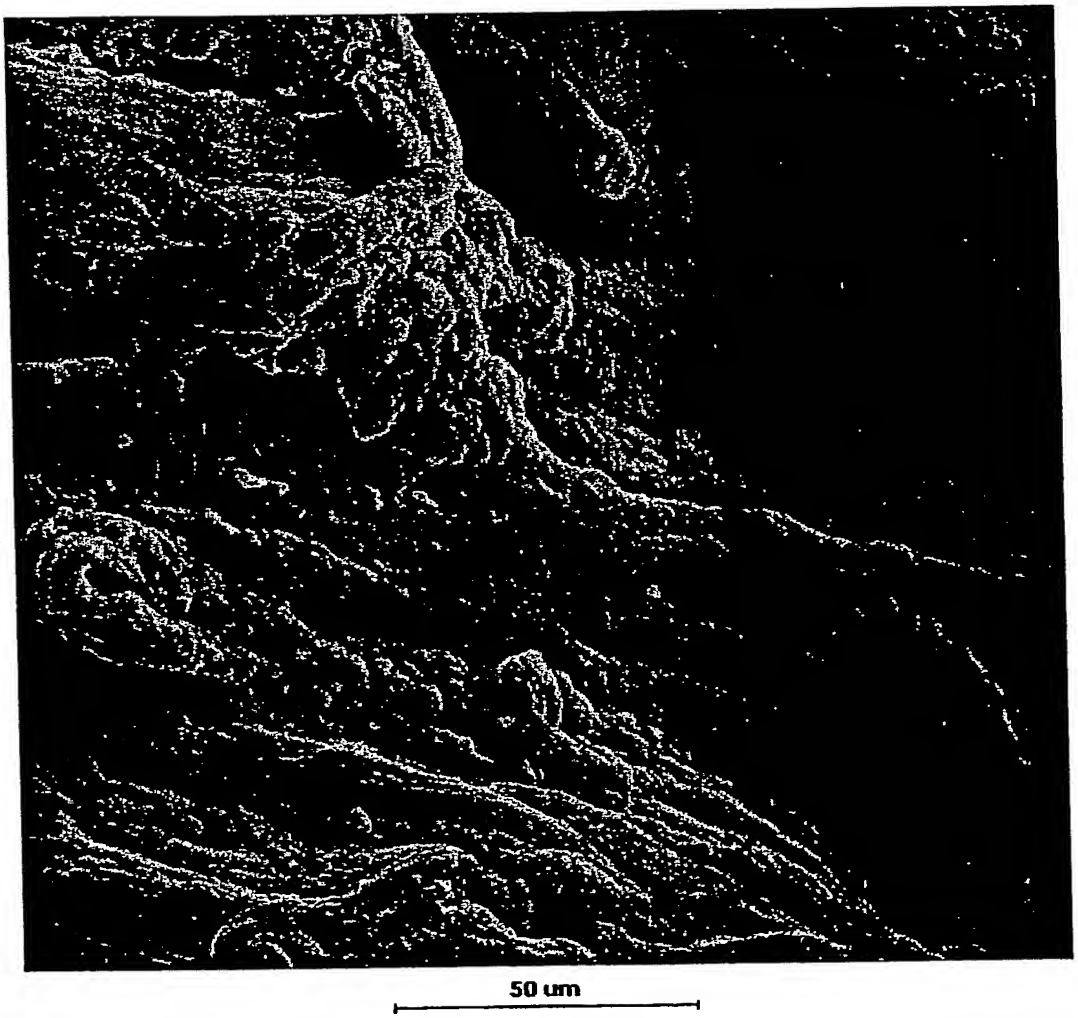

Figure 2

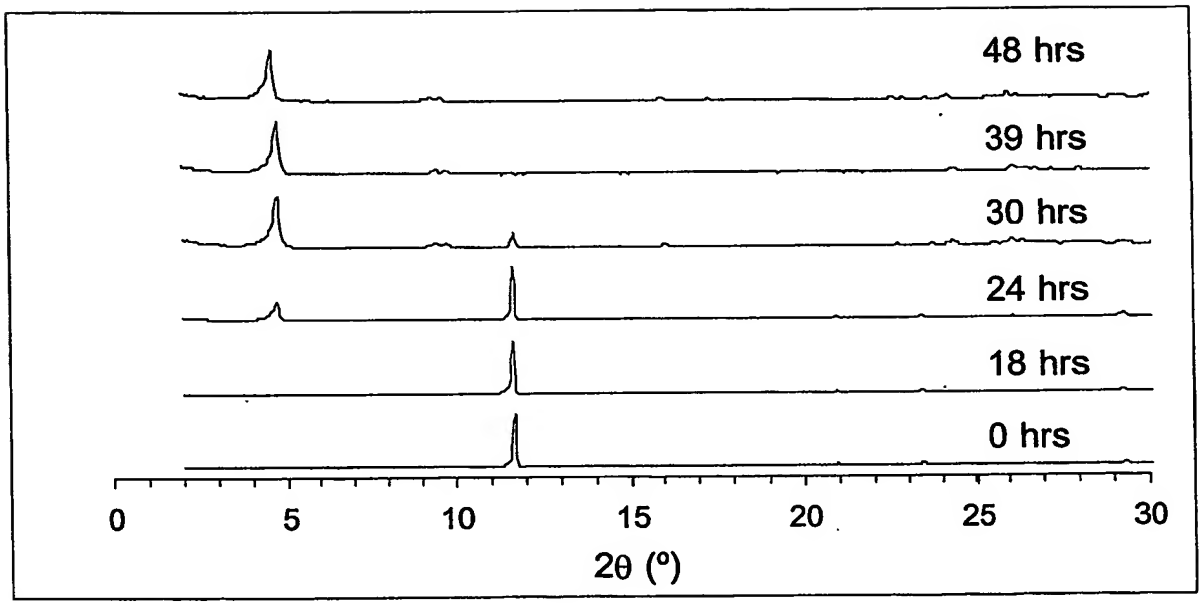

Figure 3

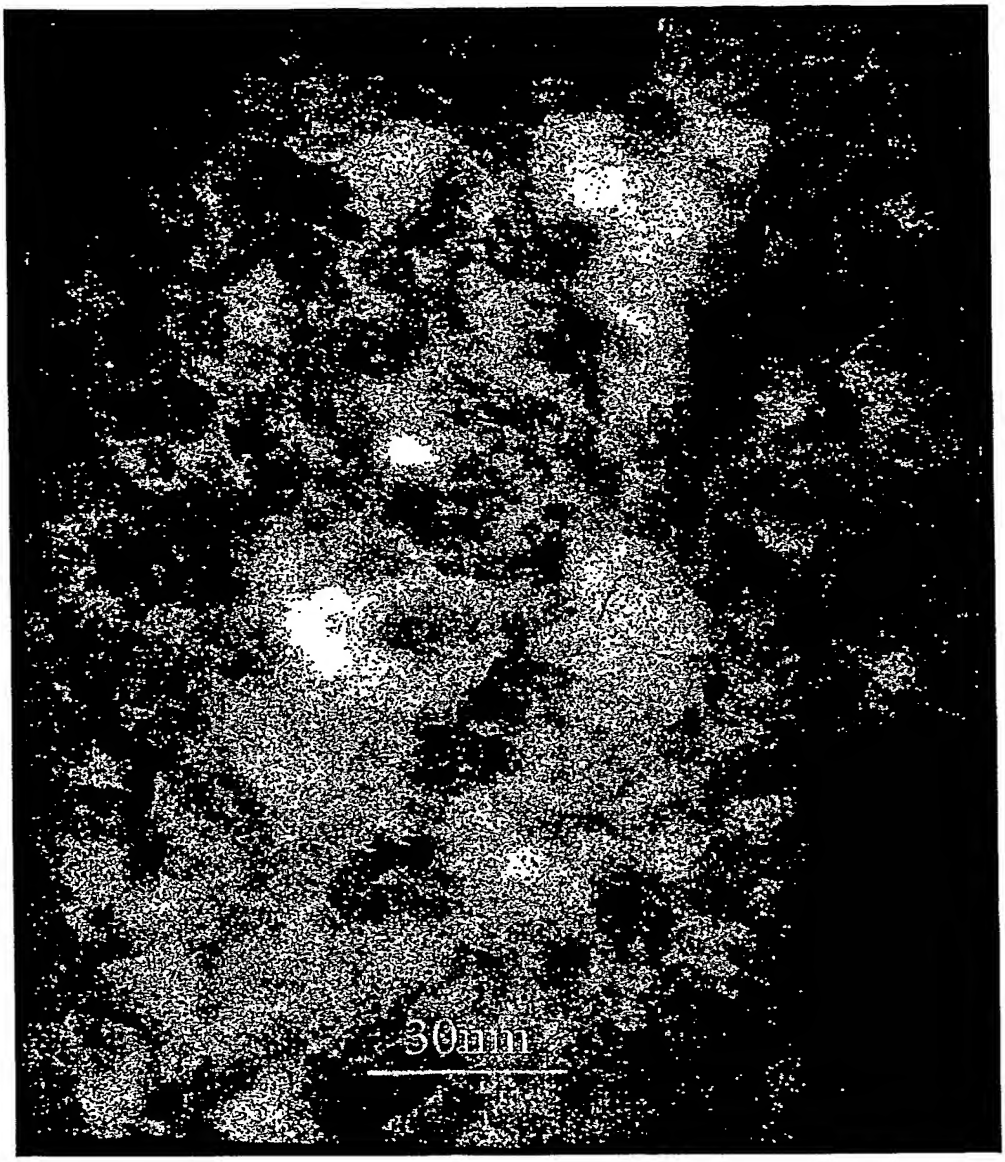

Figure 4

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